Abstract:

Testing of a culture protocol for converting mouse embryonic stem cells into neurons

Embryonic stem cells (ESCs) are pluripotent cells that are present in the inner cell mass of blastocyst-stage embryos. They are pluripotent in that they are able to differentiate into all derivatives of the primary germ layers, including ectoderm, mesoderm, and endoderm, thus generating every cell type in the body. Directed differentiation of ESCs into the cell line of interest can help to replace cells lost due to injury or disease.

The objective of this study was to culture mouse embryonic stem cells with retinoic acid to achieve neuronal differentiation and to confirm neural conversion using immunocytochemistry.

This protocol, referred to as the 4-/4+ protocol, involves spontaneous differentiation of mESCs into embryoid bodies in suspension culture for 4 days and addition of 0.5 µM RA on the 4th day. The cells were allowed to aggregate in the presence of RA for another four days and then plated on laminin-coated dishes on the 8th day. They were plated both as intact aggregates and post-disassociation with trypsin. In both cases, it was found that they gave out flat cells tightly adherent to the surface with an initial spindle shaped bipolar morphology that gradually progressed to pyramidal shaped

cells with multiple neurites. . Immunocytochemistry was used to confirm the presence of neurons by using a neuron specific isoform of monoclonal βIII-Tubulin.

The results indicate that mouse embryonic stem cells in the presence of retinoic acid differentiate into cells that phenotypically resemble neurons. These cells express the neuronal marker, βIII-Tubulin , confirming that they are neurons.

Keywords: Mouse embryonic stem cells (mESCs), Retinoic Acid (RA), differentiation, culture, neurons, βIII-Tubulin.